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博 士 学 位 论 文

生物标志物综合评价近岸海洋环境质量的研究

Study on the Evaluation of Environmental Stress in Xiamen Coastal Waters Using Multiple Biomarkers

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摘 要

本文以翡翠股贻贝 *Perna viridis* 和网箱养殖鱼类（真鲷 *Pagrosomus major* 和鮸状黄姑鱼 *Nibea miichthioides*）为指示生物，以福建东山东沈的金銮湾为参比站位，通过采集本地种和移植试验，采用酶学、微核率、生长和代谢产物等多个生物标志物，综合评价了厦门附近海域（火烧屿，厦门马銮湾内、厦门马銮湾外、同安湾）的环境质量状况。应用 SPSS 统计软件对各项化学和生物标志物参数进行相关性和主成分分析，在此基础上对研究海区的综合环境质量进行系统聚类，探讨了这些生物标志物指示不同类型环境污染状况的可行性，并对该海域环境胁迫做出总体评价。

研究表明，海洋生物抗氧化酶的变化是一种有意义的生物标志物，对于监测伴随氧化胁迫的外源性污染物早期污染，建立海洋环境的早期“警报系统”，具有一定的应用前景。胆汁中代谢产物的测定，方法简便快速，可较好的指示 PAHs 污染的近期暴露，有非常好的特异性，对于监测水体的 PAHs 污染及生物可利用性有着良好的应用前景。肝脏 EROD 活力与胆汁中 1－羟基芘的含量显著正相关，可在一定程度上说明该海域 PAH 污染物的暴露水平及生物效应。在特定污染水域的监测中，微核试验可监测环境中有遗传毒性污染物的存在，具有一定的特异性。贻贝生长余力作为一种非特异性生物标志物，可较好的说明生物生存环境的综合质量状况，有很好的应用前景。本研究通过对大量的现场生物监测和化学监测数据进行比较，提取主成分并进行聚类分析，有效的从大量纷繁复杂的数据中找到有价值的信息，客观综合地评价了调查海域的环境质量。

关键词：生物标志物；综合监测；海洋污染

Abstract

Using different kind of biomarkers (enzymatic, metabolic, cytogenetics and physiological biomarkers), this study evaluated the quality of coastal waters around Xiamen (Huoshao, IMaluan, OMaluan, Tong'an with Dongshan as a reference site) by sampling native or transplanted mussels (*Perna viridis*) and marine caged fishes (*Pagrosomus major* and *Nibea miichthioides)*. The correlation and principle factors among all chemical and biological parameters were statistically analyzed by SPSS. Base on these results, the general environmental quality of these marine areas were clustered systematically. The potential usage of biomarkers to indicate different types of pollution and the general stress in these marine areas is discussed.

The results indicated that, the variations of antioxidant enzymes showed a certain degree related to the environmental stress. The CAT responded to stress was variant with tissue. Antioxidant enzyme activities are effective toxicological markers, which can detect the effects of pollutant exposure and can be used as early-warning signals. Micronucleus assay may be a biomarker of genetic-toxically poison. Results indicated the levels of contaminants with hereditary toxicity were not high in study areas, yet the potential risk should not be ignored. The scope for growth (SFG) of *Perna v.,* as a general biomarker is promising to indicate the integrated environmental status in which organisms are living. From the view of SFG, *Perna v.* in Huoshao and Dongshen were exposed to middle stress degree**;** Stress in Maluan Bay was between high and middle degree, and closer high stress. These results indicated that SFG was an effective and economic biomarker to assess the environmental stress. The measured method of 1-OH pryene is very simple and a positive correlation between EROD activities in liver and 1-OH Pyene concentrations in bile of *Nibea m.* (or *Pagrosomus m.*) were set up in this paper, which could indicate pollution levels of PAHs and its bioavailability.

Principle components were calculated from the studied biomarker and pollutant parameters. Next, the Hierarchical cluster method was used to evaluate the integrated quality of the four marine areas. The results showed that, the water quality in Dongshan, as a referenced site, was the best and significantly different from the other sites. Among three sites around Xiamen city, the surface and deep water in Maluan were in heavy stress. In Huoshao area only deep water was highly polluted, while in Tong’an the quality of the surface water was worse. To deal with lots of data collected from field investigations, we used principle components and hierarchical cluster methods in this study. These methods effectively extract useful information and help us to draw correct conclusions.

Key words: biomarkers; integrated monitoring; marine pollution.

表 6－1　4 个站位表层水、间隙水、沉积物及生物体内不同组织多环芳烃（PAHs）、六六六（HCHs）和 DDTs 的浓度。

Table 6－1　concentrations of s PAHs, HCHs, DDTs , and organophosphate in surface water, pore water, sediment and different tissues of green-lipped mussels and *Nibea miichthioides* from four sites, 1999.

Site	Surface water(ng/L)			Sediment(ng/g)			Green-lipped mussels(ng/g dry weight)				Fish	
							whole body			Visceral	Liver	
	PAHs	HCHs	DDTs	PAHs	HCHs	DDTs	PAHs	DDTs*	Organo-phosphate*	PAHs	DDTs*	Organo-phosphate*
Dongshan	412.5	2.9	6.8	49.6	ND	3.8	/	56.96	ND	18	222.6	ND
Huoshao	36.0	ND	1.0	42.5	ND	0.6	/	114.86	ND	85	1832.8	ND
I'Maluan	71.6	1.0	6.4	96.9	ND	0.3	50	141.80	ND	45	50.0	ND
Tong'an	32.8	6.8	43.9	56.8	0.1	17.5	20	172.00	ND	64	1397.0	ND

*: ng/g wet weight (Klumpp 等，2002a;b)

ND 为低于方法检测限

表 6-2　移植后 84 天真鲷和鮸状黄姑鱼胆汁内 1-羟基芘浓度与肝脏 EROD 活力和鱼体条件指数的皮尔森相关系数。

Table6-2　Pearson Correlation coefficients between 1-OH pyrene in bile and EROD activities in liver and condition factor(CF)of two Marine fish caged fish (*Nibea miichthioides,* N; *Pagrosoma major,* P) transplanted 84days after.

	Npyrene	Nerod	Nlength	Nweight	NCF	Ppyrene	Perod	Plength	Pweight
Npyrene									
Nerod	.614(**)								
Nlength	-.014	-.018							
Nweight	-.132	-.223	.946(**)						
NCF	-.282	-.514(**)	-.181	.124					
Ppyrene	.318	.365	.322	.011	-.753(**)				
Perod	.612(**)	.970(**)	.131	-.088	-.559(**)	.480(*)			
Plength	-.343	-.068	.301	.209	-.287	.283	-.080		
Pweight	-.418	-.254	.345	.262	-.314	.223	-.249	.840(**)	
PCF	-.149	-.359	-.029	-.009	-.020	-.187	-.325	-.435(*)	.115

**　Correlation is significant at the 0.01 level (2-tailed).

*　Correlation is significant at the 0.05 level (2-tailed).

表 6-3　4 个站位翡翠股贻贝各生物标志物

Table 6-3　Values of each biomarkers of green-lipped mussel (*Perna viridis*) from four sites, 1999.

Site	Biomarkers ATP(G)(μmol.min.mg^{-1}) (n=80)	ATP(V)(μmol.min. mg^{-1}) (n=80)	CAT(G) (U.mg^{-1}) (n=80)	CAT(V) (U.mg^{-1}) (n=80)	SOD(G) (U.mg^{-1}) (n=80)	SOD(V) (U.mg^{-1}) (n=80)	SFG(n=64) (Jg^{-1}h^{-1})	MN(‰) (n=40)
Dongshan	11.10±2.77	3.15±1.41	23.89±13.84	24.99±10.61	5.20±3.24	7.38±2.61	10.95±2.95	1.97±0.37
Huoshao	10.53±4.04	2.69±1.27	21.92±10.94	32.65±10.68	3.84±0.95	4.60±1.10	6.72±2.60	1.23±0.42
IMaluan	10.66±3.78	2.63±1.20	6.25±2.98	177.19±64.81	3.34±3.30	2.80±0.50	5.10±3.22	2.00±0.44
Tong’an	12.35±3.72	3.65±0.74	5.77±1.97	140.95±22.69	4.60±1.47	3.60±1.68	1.12±2.12	2.20±0.39

G: gill; V: visceral mass

表 6-4　4 个站位鮸状黄姑鱼各生物标志物

Table 6-4　Values of each biomarkers of fish (*Nibea miichthioides*) from four sites, 1999.（Klumpp 等[132,184]）

Site	biomarkers EROD(nmol. min^{-1}.mg^{-1})	GST(pmol.min^{-1}.mg^{-1})	GSH (μg.g^{-1})	GPx(nmol.min^{-1}.mg^{-1})	SOD(U. mg^{-1})	CAT(μmol .min^{-1}.mg^{-1})	AChE(μmol. min-1.mg01)	Naphalene metabolites(ng.g^{-1})	Phananthrene metabolites(ng.g^{-1})	1-OH pryene* (ng.ml^{-1})
Dongshan	7	520	38	68	38	1.5	3.1	490	125	234.68
Huoshao	18	650	78	97	50	1.8	2.2	400	80	224.02
IMaluan	16	440	39	88	39	1.6	2.9	395	135	295.20
Tong'an	13	700	56	70	59	2.2	2.5	180	40	197.41

*This paper.

表 6-5　从东山移植到厦门后各个站位翡翠股贻贝鳃和内脏团 CAT 和 SOD 活力

Table 6-5　CAT and SOD activities in gill and ves of green-lipped mussel (*Perna viridis*) in three sites around Xiamenafter transplanted from Dongshan, 2000.

Site	CAT ($U.mg^{-1}$)								SOD ($U.mg^{-1}$)							
	Gill				Visceral mass				Gill				Visceral mass			
	7	28	56	84	7	28	56	84	7	28	56	84	7	28	56	84
Dongshan	/	/	/	11.47				288.86				0.65				1.23
Huoshao	10.99	18.01	20.7	19.54	59.34	61.19	106.92	416.29	1.75	1.92	1.52	1.21	2.56	1.59	2.79	2.51
IMaluan	15.17	12.60	33.12	22.54	126.23	187.1	210.4	306.73	2.62	1.12	0.94	0.76	1.40	1.93	1.68	1.5
O'Maluan	13.13	14.89	37.11	19.03	66.74	84.33	173.4	314.27	2.92	2.28	1.22	0.85	2.37	3.50	2.69	2.45

表 6-6　从东山移植到厦门后各个站位两种鱼（真鲷和鮸状黄姑）胆汁中 1-羟基芘浓度及肝脏 EROD 活力

Table 6-6　1-OH pyrene concentrations in bile and EROD activities in liver of two fish (*Pagrosoma major* and *Nibea miichthioides*) from three sites around Xiamen after transplanted from Dongshan, 2000.

Site	*Nibea miichthioides*				*Pagrosoma major*			
	1- OH pyrene ($ng.ml^{-1}$)		EROD($pmol.min^{-1}.mg^{-1}$)		1- OH pyrene ($ng.ml^{-1}$)		EROD($pmol.min^{-1}.mg^{-1}$)	
	28days	84 days	28 days	84 days	28 days	84 days	28 days	84 days
Dongshan		348.29		2.12		201.53		7.22
Huoshao	507.63	763.84	5.43	6.21	417.06	452.52	40.25	10.33
IMaluan	512.52	1476.27	29.61	34.64	509.03	445.93	37.14	80.73
O'Maluan	576.82	466.75	5.38	2.60	664.46	301.64	38.90	4.56

表 6-7　翡翠股贻贝各生物标志物及软体组织内 DDTs 浓度间的皮尔森相关系数

Table 6-7　Pearson correlation coefficients between DDTs and biomarkers in green-lipped mussel (*Perna viridis*) .

	SFG	MN	GATP	VATP	GCAT	VCAT	GSOD	VSOD	DDT
SFG	1.000								
MN	-0.326	1.000							
GATP	-0.602	0.684	1.000						
VATP	-0.400	0.631	0.970*	1.000					
GCAT	0.852	-0.621	-0.472	-0.252	1.000				
VCAT	-0.737	0.599	0.299	0.077	-0.976*	1.000			
GSOD	0.352	0.338	0.525	0.715	0.429	-0.528	1.000		
VSOD	0.832	-0.112	-0.092	0.153	0.847	-0.845	0.799	1.000	
DDT	-0.987*	0.274	0.477	0.255	-0.880	0.793	-0.493	-0.909	1.000

*　Correlation is significant at the 0.05 level (2-tailed). G: gill; V: visceral mass

表 6-8　鮸状黄姑鱼各生物标志物及肝脏内 DDTs 浓度间的皮尔森相关系数

Table 6-8　Pearson correlation coefficients between DDTs and biomarkers in fish, *Nibea miichthioides*

	EROD	GST	GSH	GPx	SOD	CAT	AChE	Naphalene metabolities	Pananthrene metabolities	1-OH Pyrene	DDTs
EROD	1.000										
GST	0.235	1.000									
GSH	0.612	0.742	1.000								
GPx	0.796	-0.080	0.586	1.000							
SOD	0.427	0.931	0.639	-0.056	1.000						
CAT	0.410	0.848	0.487	-0.147	0.979*	1.000					
AChE	-0.738	-0.773	-0.967*	-0.565	-0.760	-0.648	1.000				
Naphalene metabolities	-0.409	-0.669	-0.267	0.212	-0.885	-0.960*	0.475	1.000			
Pananthrene metabolities	-0.315	-0.963*	-0.629	0.138	-0.991**	-0.959*	0.727	0.845	1.000		
1-OH Pyrene	0.151	-0.925	-0.525	0.383	-0.774	-0.694	0.503	0.508	0.852	1.000	
DDTs	0.508	0.910	0.952*	0.333	0.828	0.701	0.954*	-0.490	-0.832	-0.733	1.00

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

表 6-9　表层水（S－）、沉积物（D-)、生物体（贻贝整体，MB-；贻贝内脏团，MV-；鱼肝脏，FL-）内 PAHs、HCHs 和 DDTs 浓度与各生物标志物的皮尔森相关系数

Table 6-9　Pearson correlation coefficients between DDTs, HCHs, DDTs in surface water(S-), pore water(P-), sediment(D-), and biota(mussel whole body, MB-;mussel visceral, MV-; fish liver, FL-) and biomarkers in fish, *Nibea miichthioides*(F-) and green lipped mussels(M-).

	S－PAH	S－HCH	S－DDT	D－PAH	D－DDT	MB－DDT	MV－PAH	FL－DDTS
S－PAH	1.000							
S－HCH	.007	1.000						
S－DDT	-.302	.951*	1.000					
D－PAH	-.237	-.139	-.053	1.000				
D－DDT	-.197	.970*	.985*	-.214	1.000			
MB－DDT	-.880	.403	.658	.385	.539	1.000		
MV－PAH	-.860	-.135	.134	-.253	.110	.584	1.000	
FL－DDTS	-.574	.105	.272	-.659	.327	.353	.881	1.000
F-Naphalene metabolities	.665	-.742	-.912	-.023	-.861	-.882	-.492	-.490
F-Pananthrene metabolities	.534	-.627	-.759	.501	-.793	-.592	-.656	-.832
F-PYRENE	.041	-.609	-.588	.836	-.705	-.094	-.348	-.733
F-EROD	-.954*	-.308	.002	.277	-.105	.718	.854	.508
F-GST	-.411	.493	.592	-.706	.665	.380	.667	.910
F-GSH	-.586	-.204	-.018	-.589	.026	.243	.917	.952*

续上表

	S－PAH	S－HCH	S－DDT	D－PAH	D－DDT	MB－DDT	MV－PAH	FL－DDTS
F-GPX	-.581	-.811	-.594	.146	-.655	.155	.657	.333
F-SOD	-.640	.590	.757	-.399	.768	.688	.714	.828
F-CAT	-.651	.688	.855	-.239	.842	.782	.624	.701
F-ACHE	.759	.074	-.160	.433	-.170	-.483	-.981	-.954
M-ATPG	-.097	.993**	.976*	-.175	.992**	.476	-.014	.215
M-ATPV	.111	.975*	.894	-.348	.951*	.255	-.125	.201
M-CATG	.609	-.453	-.624	-.705	-.479	-.880	-.145	.126
M-CATV	-.548	.294	.454	.841	.294	.793	.046	-.287
M-SODG	.721	.579	.326	-.624	.466	-.493	-.502	-.037
M-SODV	.897	-.027	-.306	-.631	-.151	-.910	-.550	-.162
M-SFG	.829	-.531	-.763	-.274	-.664	-.987*	-.566	-.407
M-MN	.211	.751	.655	.451	.603	.274	-.588	-.549

** Correlation is significant at the 0.01 level (2-tailed).

Correlation is significant at the 0.05 level (2-tailed).

缩略词表

缩略词	中文	英文
ACHE	乙酰胆碱酯酶	Acetylcholin
AHH	芳香烃羟基化酶	Arylhydrocarbon hydroxylase
BAM	生物富集监测	Bioaccumulation monitoring
BEM	生物效应监测	Biological effects monitoring
CAT	过氧化氢酶	Catalase，
CM	化学监测	Chemical monitoring
EM	生态监测	Ecosystem monitoring
EROD	7-乙氧基异吩恶唑酮-脱乙基酶	Ethoxyresorufin O-deethylase
GRED	谷胱甘肽还原酶	Glutathione reductase
GPx	谷胱甘肽过氧化物酶	Glutathione peroxidase
GSH	还原型谷胱甘肽	Reduced glutathione
GSSG	氧化型谷胱甘肽	Oxidized glutathione
GSTs	谷胱甘肽硫转移酶系	Glutathione S-transfereases
HM	健康监测	Health monitoring，
HCHs	六六六	Hexachlorocyclohexane
HSPs	热激蛋白	Stress proteins
NRC	国家研究委员会	National Research Council
MFO	混合功能氧化酶	Mixed-function oxidase,
MTs	金属硫蛋白	Metallothioneins
MXR	多重异生物抗性	Multixenobiotic resistance
PAHs	多环芳烃	Polycyclic aromatic hydrocarbons
PCBs	多氯联苯	Polychlorinated biphenyls
POPs	持久性有机污染物	Persistant organic pollutants
QTI	定量毒性解释	Quantitative toxicological interpretation
SFG	生长余力	Scope for growth
SOD	超氧化物歧化酶	Superoxide dismutase
UDPGTs	尿苷二磷酸葡萄糖醛酸基转移酶系	UDP-glucuronyl transferases
WHO	世界卫生组织	World health organization
Na	萘	Naphthalene
Ace	苊	Acenaphthylene
Acen	苊烯	Acenaphthene
Flu	芴	Fluorene
Phen	菲	Phenanthrene
An	蒽	Anthrcene
Fluo	荧蒽	Fluoranthene

Py	芘	Pyrene
BaA	苯并[a]蒽	Benzo(a)anthracene
Chr	屈	Chrysene
BbF	苯并[b]荧蒽	Benzo(b)fluoranthene
BkF	苯并[k]荧蒽	Benzo(k)fluoranthene
BaP	苯并[a]芘	Benzo(a)pyrene
Indeno	茚哚芘	Indeno(1,2,3-cd)pyrene
DbA	二苯并[a]蒽	Dibenzo(a,h)anthracene
BgP	苯并[ghi]北	Benzo(ghi)perylene

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